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Influence of food supply on population structure and length distribution in *Acrobeloides nanus* (Nematoda: Cephalobidae)

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With 4 figures

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Contents

1. Introduction

There has been some discussion about the amount of food available for bacterial feeding nematodes in the soil. When the number of bacteria and nematodes are compared it seems clear that there is an enormous amount of potential food for these nematodes (RUSSELL 1957, BANAGE and VISSER 1967). It is however not known what proportion of the bacterial populations is available to the nematodes. It is also uncertain whether the so called bacterial feeding nematodes eat bacterial cells only (YEATES 1971). To this may be added that we know little about the ingestion rate of these animals.

One indirect way of getting some information about the food supply in soil for these animals is to observe if they appear to be well-fed or not. Thus the adult body length might decrease during periods of starvation (FISHER 1969). Also the population structure and the frequency of gravid females may be influenced by the food supply. This is indicated from studies of populations of rhabditid nematodes cultured on agar (SOHLENIUS 1969a and b). In these cultures the structure of fresh expanding populations differed much from the structure of populations from old cultures with increasing food shortage. Thus if soil populations are compared with laboratory populations of different ages it might be possible to conclude if the field populations are starving or not. This is the basic idea in the present study in which laboratory reared and field populations of an uniparental species of *Acrobeloides* [probably *A. nanus* (DE MAN 1880)] are compared.

The growth and reproduction of *A. nanus* when cultured on agar have been described in an earlier paper (SOHLENIUS 1973).

2. The study area

A field population of *A. nanus* from a Swedish pine forest soil was studied. The locality is situated in the Rösjö-reservation about 20 km north of Stockholm. The tree layer consists of common fir (*Picea abies*) and Scotch fir (*Pinus silvestris*) and the principal component of the ground layer is *Vaccinium myrtillus*, which is mixed with mosses and grasses (*Deschampsia* spec.). The soil profile is characterised by a distinct raw humus layer (pH 4–5) about 2–5 cm in thickness. Temperature was measured in the raw humus layer at a depth of 2–3 cm below the surface (Table 1) in connection with samplings (1–4 measurements each month).

Table 1 Temperatures in the raw humus layer 2–3 cm below surface (°C)

Year	Month											
	J.	F.	M.	A.	M.	J.	J.	A.	S.	O.	N.	D.
1969	—	—	—	—	—	14.7	16.0	13.1	10.7	6.0	3.8	2.0
1970	0.5	0.2	0.2	0.4	7.0	13.6	13.4	11.8	8.4	7.0	3.7	—

3. Methods

3.1. Sampling and extraction

The population was randomly sampled every month from April 1969 to March 1970. The number of units per sample was usually 12, but during the autumn the number was increased to 15–18 and in August the number was 6. Soil blocks about 250 *ml* were cut out. In the laboratory a 2.8 *ml* subsample was taken from the raw humus layer.

The animals were extracted in modified Baermann funnels by a method similar to that used by Nielsen (1948) and Banage (1966) (see Sohlenius 1969c and 1971). The extraction lasted for 24 hours. The animals were killed by heat and fixed in F.A. 4 : 1 (A water solution with 4 per cent formalin and 1 per cent acetic acid).

3.2. Laboratory studies

Nematodes from the locality were cultured in Nigon's agar (Nigon 1949), modified according to Sohlenius (1968), with *Escherichia coli* (Migula 1895) as a food organism. The same strain of *A. nanus* as was isolated for the previous study (Sohlenius 1973) was used.

In order to study the development of laboratory populations each of five agar plates (diameter: 9 cm) containing 25 *ml* agar was inoculated with 5 young gravid females. The bacteria were transferred together with the inoculation animals. At different times after inoculation the population density, the frequency of gravid females and the composition of length classes were examined. For this two small agar plugs, 6 mm in diameter, from each culture were removed (for details see Sohlenius 1969a).

The effects of starvation upon adult body length and the frequency of gravid specimens were studied on animals that had been kept in salt solution for some different times. The animals used in these experiments had first been cultured in Nigon's agar until an age of eleven days. After this they were transferred to glass dishes with salt solution of the same composition as in the agar medium ($NaCl$ 1.375, KNO_3 1.5, $MgSO_4$ 0.375 and K_2HPO_4 0.375 g in 1000 *ml* water).

All laboratory experiments were performed at a temperature of 21 °C in darkness.

3.3. Field experiment

An experiment was also undertaken in order to study the effect of the enrichment with organic material upon the nematodes in field. In this experiment boiled fungal fruiting bodies (from the study area) and faeces (human) were used. The material was subdivided in lumps of about 100 ml each. These were placed in bags of nylon-netting (mesh size 0.7 mm). Five bags with fungal material and 2 with faeces were buried in the soil so that they were covered with soil but still in contact with the raw humus layer. At different times after their application quantitative samples (2.8 ml) were taken from the soil close to the bags and non-quantitative samples were taken from the interior of the bags. This experiment was started on October 14th, 1969 and was continued until about the 20th of November the same year.

4. Results and discussion

4.1. The field population

In most samples from field *Acrobeloides nanus* was the most common species among the bacterial feeding nematodes. Over the whole sampling period it constituted 9.6 per cent of the entire nematode population and was found in 84.7 per cent of the sample units. The population between April 1969 and March 1970 fluctuated between 3.7 and 16.9 with an annual mean population of 9.6 specimens per *ml* soil. The confidence limits in Fig. 1a are very wide which indicates that the population was quite aggregated. The lowest number of animals was obtained during the summer and the highest during the autumn. Gravid females were found in May, September to December and in March (Fig. 1b). The total number of adult females was less variable and thus all the examined females were devoid of eggs during certain months. However in June no adults were found and in July they were 2 per cent.

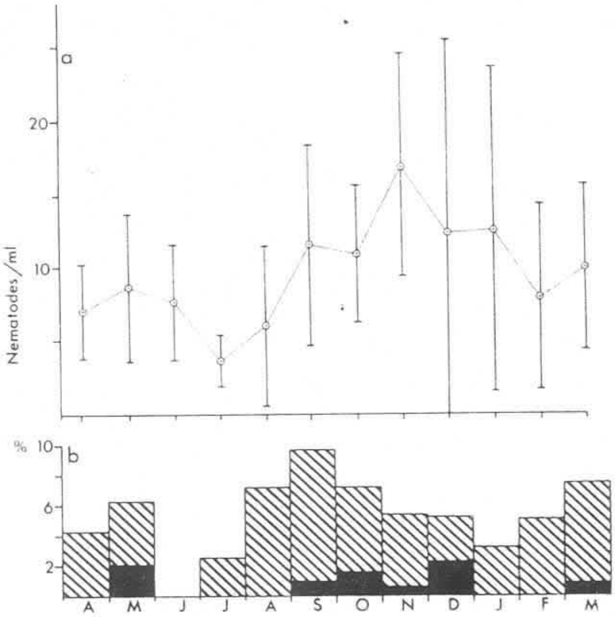

Fig. 1a. Fluctuation in the mean population of *Acrobeloides nanus* (mean number per ml with 90 % confidence limits) between April 1969 and March 1970. b. The percentage of non-gravid females (striped) and gravid females (solid) in the *A. nanus* population.

The generation time for *A. nanus* is about 11 days at 21 °C and 21 days at 13 °C (SOHLENIUS 1973). Therefore it seems reasonable to assume that the field temperature (Table 1) might permit at least 6 generations to be produced during the year. However it is probable that the growth rate in field is not solely dependent upon temperature. The increase in frequency of adults from July to September indicates that the individuals were maturing during this period. Apparently the chief period of recruitment to the population took place during the autumn. This conclusion is supported by the increase in frequency of gravid females, the increased population density and also by the high proportion of short specimens in October and November (Fig. 2). From Fig. 2 it is however

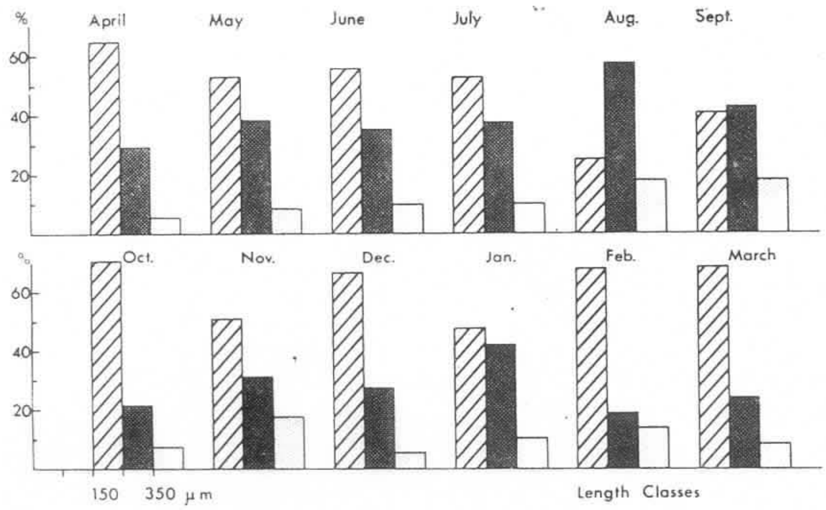

Fig. 2. Length class representation in the population of *Acrobeloides nanus* between April 1969 and March 1970.

evident that the population length structure did not change very much through the year with the exception that the frequency of short specimens was strikingly lower in August and September samples.

4.2. Laboratory reared populations

Agar cultures were started as was described in section 3.2. The cultures were examined at 18, 25, 31, 38, 45, 53, 59 and 66 days after inoculation. The rate of increase in nematode number was slow at first (Table 2). Later on the growth rate increased markedly and the highest rate (approx. 5× in 7 days) was obtained between 31 and 38 days after inoculation. The peak density was obtained at 53 days with about 730 animals per plug (183,000 per plate). High densities prevailed for rather long periods and over 500 nematodes per plug were obtained from 38 days until the experiment ended.

Table 2 The development of bacteria and nematodes in monoxenic agar cultures (*E. coli* on Nigon's agar). Each culture was started with five gravid females of *Acrobeloides nanus*

Days since inoculation	Number per plug[1])			Frequency of gravid females (%)	Bacterial[2]) growth
	Nematodes	Eggs	Gravid females		
0	0.02	0.0	0.02	100.0	—
18	30.3	27.9	3.0	10.0	++
25	48.9	16.9	1.2	2.4	+++
31	108.1	68.9	9.9	9.2	+++
38	558.3	362.2	22.9	4.1	+
45	697.5	241.2	0.0	0.0	—
53	728.8	209.6	5.8	0.8	—
59	654.9	110.8	0.0	0.0	—
66	521.0	95.5	0.0	0.0	—

1) Each figure is the mean of 2 plugs from each of 5 cultures.
2) +++ extensive, ++ moderate, + slight, — no visible bacterial growth.

Table 3 Influence of starvation upon body length and frequency of gravid specimens in laboratory reared adult females of *Acrobeloides nanus*

	No. observed animals	Length (μm)		S. E.	Frequency of gravid specimens (%)
		Mean	Range		
Well-fed 9–15 days old	54	525.6	480–553	2.33	68.0
Well-fed					
11 days old	10	529.7	506–549	4.28	80.0
4 days starvation	9	505.1	485–520	3.91	33.0
8 days starvation	13	494.9	472–516	4.33	7.7

The visible bacterial growth had not disappeared until between 38 and 45 days after inoculation (Table 2) and it seems likely that the food supply was limiting from 38 days. Thus the histograms from the first three readings (18 to 31 days) come from populations which have a surplus of food at their disposal (Fig. 3).

The high bar of the shortest length class at 18 days contains largely juveniles produced by the offspring of the inoculation animals. As the generation time at 21 °C is about 11 days (Sohlenius 1973) these juveniles had not matured at 25 days but had at 31 days. This is reflected in the low frequency of gravid females at 25 days and the high frequency at 31 days (Table 2). One of the causes of the high rate of increase in nematode number between 31 and 38 days is the high frequency of gravid adults at 31 days.

After 38 days the effects of the increasing shortage of food upon the number of gravid females, the number of eggs and the composition of length classes become noticeable. The number of gravid females after 38 days is obviously very small. Therefore it seems strange that the number of eggs remains so high. The cause of this must be either that there is a delayed hatching or that many of the eggs laid in old cultures are infertile. Certainly the recruitment to the population decreases drastically and probably soon ceases altogether. This development together with the relatively stable population structure and rather

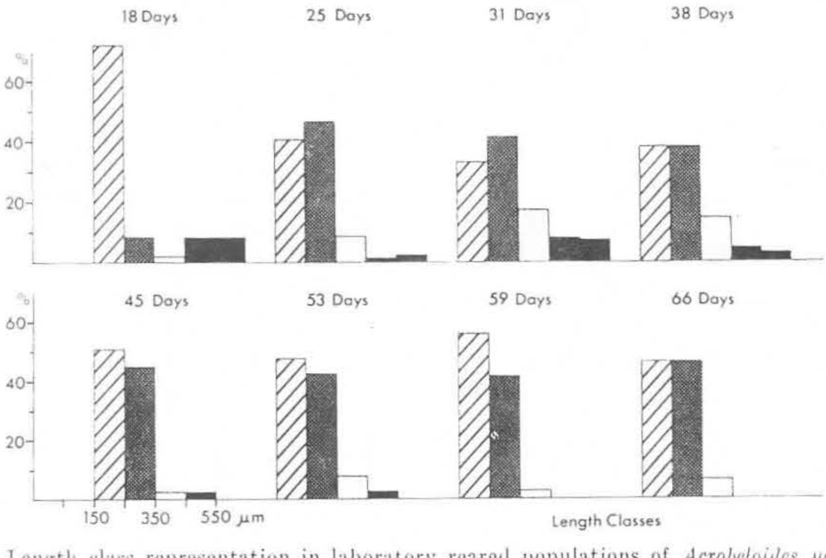

Fig. 3. Length class representation in laboratory reared populations of *Acrobeloides nanus* at different times after inoculation.

constant population density indicates that the population becomes almost static. After 45 days the same specimens are to a great extent found in the same length classes during succesive readings. Thus there is a slight or no growth of the individuals. The decrease in frequency and later the disappearence of animals in length classes over 450 μm are an effect of mortality and probably also of shrinkage.

4.3. Influence of starvation upon adults

From Table 3 it is evident that after transfer to salt solution the body length and the frequency of gravid specimens gradually decreased. After the transfer to the salt solution at an age of 11 days the animals were 34.8 μm (6.6 per cent) shorter in 8 days. As a comparison values are included which are obtained from measurements of 9–15 days old well fed females. It was found that these were definitely longer than those exposed to starvation.

The frequency of gravid females decreased drastically and was about 10 times lower after 8 days of starvation (Tab. 3).

4.4. Field experiment

After the application of bags with organic material higher numbers of *A. nanus* were obtained in the soil adjacent to these bags than in the untreated soil (Table 4). However these differences were not significant ($p > 0.05$) for the fungal material and the figures from the bags with faeces were not tested due to the few replicates.

On the first examination *A. nanus* was found inside the enrichment material in all bags except in one containing faeces. On the second examination however it was found only in one of the fungal bags and on later examination *A. nanus* was not found in any of the bags. This might be an effect of competition from the rapidly expanding *Rhabditis* populations.

Table 4 Effect of the application of bags with organic material upon number of *Acrobeloides nanus*. The figures show number from soil adjacent to the bags

Days since start	Number of nematodes per ml soil				
	Faeces	Fungus		Untreated soil	
	Mean	Mean	S. E.	Mean	S. E.
6–8	45.1	18.0	4.9	10.8	2.7
14–16	22.2	18.8	7.1	—	—
23	21.8	57.6	28.3	—	—
35–36	48.5	39.9	17.2	16.9	4.2

Table 5 The body length of adult females and the frequency of gravid females in field populations of *Acrobeloides nanus*

	Enrichment experiment with fungal material				
	Inside the material	In the surrounding soil			Untreated soil (On occurrence)
Days since application	8	16	23	35	
Length (μm)					
Mean	437.5	420.1	407.5	400.3	382.3
Range	410–450	370–480	370–465	362–445	348–418
S.E.	8.9	9.3	12.9	13.9	7.0
n	4	16	5	6	10
Per cent gravid females	16.0	18.2	6.2	5.9	1.5

The composition of length classes in the population extracted from the soil around the bags differed already during the first readings (6 to 8 days after start) from the length class composition of the population from the untreated soil (cf. Fig. 2 Oct. and Nov. and Fig. 4).

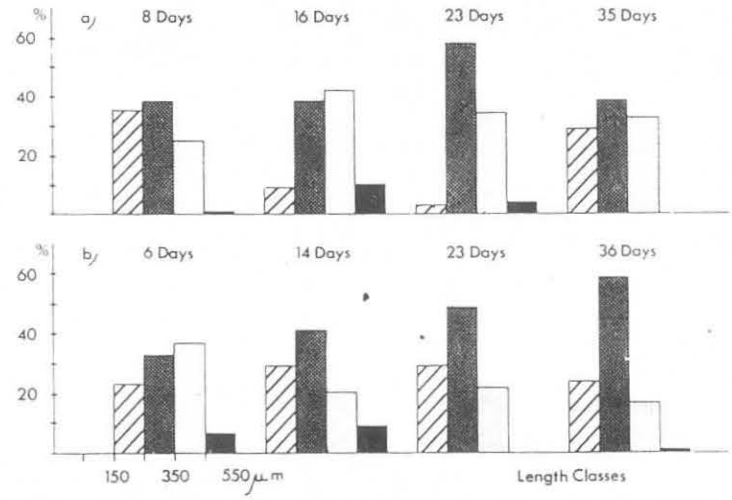

Fig. 4. Length class representation in populations of *Acrobeloides nanus* at different times after enrichment with organic material. (a) comes from animals extracted from soil close to fungus and (b) from material close to faeces.

This change of length class composition consisted intitially of a decrease of the propor tion of short animals and an increased proportion of longer ones. As can be seen on Fig. 4 there also occurred one length class which exceeded the longest length class from the untreated soil. The animals from the enrichment became thicker and their intestines were filled and opaque. There appeared adults that were longer than any of those extracted from the untreated soil (Table 5).

Another effect of the application of organic material was that the frequency of gravid females increased quite pronouncedly (Table 5).

5. Conclusions

Comparing the results from the different parts of this study it seems quite certain that the natural soil population of *A. nanus* lived under conditions of limiting food supply. This is indicated by the lengths of the females, the frequency of gravid females and by the distribution of length classes in the population.

The differences in body lengths between females from field and laboratory were quite great. Even those laboratory reared females that had been starving in salt solution were about 75 μm longer than the well fed ones from the beginning of the field experiment. Probably factors other than the amount of food also influence the size. These observations are in line with results of ANDERSON (1965 and 1968) who found that agar reared adults were longer than those reared in soil cultures. In *Acrobeloides uberrinus* (ANDERSON 1965) the agar cultured specimens were just slightly longer (40 μm) but in *A. nanus* (ANDERSON 1968) they were as much as 75 μm longer.

In the present study well-fed agar reared females were about 100 μm longer than well-fed ones obtained in the beginning of the field experiment and about 140 μm longer than those from untreated soil.

These results indicate that direct comparisons between sizes of laboratory reared females from soil may be inconvenient for conclusions regarding food supply. However the females from the beginning of the field experiment were definitely longer than those from the natural population (Table 5).

It was shown in the starvation experiment that the frequency of gravid females decreased during the starvation (Table 3). The frequency of gravid females within a population of *A. nanus* might therefore indicate if the food is short or not. Among the animals extracted over the whole year from the untreated soil 0.8 per cent were gravid or 1.5 per cent if samples in which gravid *A. nanus* occurred are considered. Among all females extracted from the soil (5.6 per cent of the population) 14.4 were gravid. These values are far below those obtained from the field experiment (Table 5), from the beginning of the agar experiment (Table 2) and from the singly reared well-fed adults in the starvation experiment (Table 3).

The length class composition of the natural population (Fig. 2) is most similar to the length class composition of the old starving agar-reared populations at 59 and 66 days (Fig. 3). This indicates that the natural population is relatively static. This conclusion is supported by the effect of enrichment upon length class composition (Fig. 4). Obviously this addition of food promoted the growth of the individuals.

6. Acknowledgements

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7. Summary · Zusammenfassung

Laboratory reared and field populations of the bacterial feeding nematode *Acrobeloides nanus* were studied. It was found that starvation lead to a reduction of adult body length and a decrease in the frequency of gravid females. The structure of the field population was most similar to the structure of old starving laboratory populations. Enrichment with organic material to the field population lead to changes in population structure. Adults from the enriched soil and from agar cultures were much bigger and contained eggs in higher frequencies than those extracted from the untreated soil. It is considered that the food supply in field was a limiting factor during the whole year. The most favourable conditions prevailed during the autumn.

(Einfluß der Nahrungsversorgung auf die Populations-Struktur und Längenverteilung von *Acrobeloides nanus*)

Hungerexperimente mit im Laboratorium gezogenen und im Freiland gehaltenen Populationen von bakterienfressenden Nematoden der Art *Acrobeloides nanus* führten zu einer Verringerung der Körperlänge der adulten und zu einer Abnahme der Häufigkeit der graviden Weibchen. Gerade solche im Laboratorium gezogenen Weibchen, die in Salzlösung gehungert haben, waren etwa 75 μm länger als die gut ernährten Weibchen zu Beginn des Freilandexperimentes. Wahrscheinlich beeinflussen auch andere Faktoren (als die Nahrungsmenge) die Körpergröße. Die Häufigkeit gravider Weibchen innerhalb einer Population von *A. nanus* mag darauf hinweisen, ob die Nahrung knapp war oder nicht. Die Struktur von Freilandpopulationen war der von alten hungernden Laborpopulationen am ähnlichsten. Ein reiches Angebot von organischem Material führte zu Veränderungen der Struktur einer Freilandpopulation. Adulte Tiere aus angereichertem Boden und von Agarkulturen waren viel größer und enthielten häufiger Eier als solche, die aus unbehandeltem Boden ausgelesen wurden. Es wurde erwogen, daß das Nahrungsangebot im Freiland während des ganzen Jahres der begrenzende Faktor sei. Die günstigsten Bedingungen herrschten während des Herbstes.

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